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LRD-GB-4-417

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0319797.7

24AUG03 0835A37-1 010050

P01/7700 0.00-0319797.7

3. Full name, address and postcode of the or of each applicant (underline all surnames)

K.U.Leuven Research and Development - Groot Begijnhof 59 - 3000 Leuven

Represented by Dr. Ivo Roelants, IPR Officer

Patents ADP number (if you know it)

08070948001

If the applicant is a corporate body, give the country/state of its incorporation

Belgium

## 4. Title of the invention

Particle size reduction of poorly soluble drugs

## 5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents ADP number (if you know it)

08007916003

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Number of earlier application

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11.

I/We request the grant of a patent on the basis of this application.

Dr. Ivo Roelants, IPR Officer

Signature

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Patents Form 1/77

## Particle size reduction of poorly soluble drugs

### FIELD OF THE INVENTION

5 The invention relates to the reduction of the size of solid drug particles in aqueous suspensions by conduction of the suspensions through magnetic fields. The particle size is reduced from the micrometer to the nanometer range. Furthermore, the invention relates to methods allowing the stabilisation of the obtained nano-particles  
10 as well as to formulations containing said stabilised nano-particles. The formulations of the present invention are of particular relevance for the oral delivery of poorly soluble drug particles.

### 15 BACKGROUND OF THE INVENTION

With the introduction of combinatorial chemistry and high throughput screening, there is likely to be no shortage of leads and hits. The molecular structures of new chemical entities are becoming more complex leading to drugs with low aqueous solubility and dissolution rate limited absorption after oral administration, still the  
20 preferred route of drug administration. Considering the fact that many newly synthesized molecules are poorly soluble in aqueous environment, converting these compounds into useful therapeutics remains challenging. Techniques that have commonly been used to improve dissolution and bioavailability of poorly water-soluble drugs in general, include micronization (Atkinson et al., 1962), the use of  
25 surfactants (Khalafalla et al., 1975), and the formation of solid dispersions (Sekiguchi and Obi, 1961). Chiou and Riegelman (1971) outlined six types of drug-carrier interactions in solid state dispersions: simple eutectic mixtures, solid solutions, glass solutions, glass suspensions, amorphous precipitates in a crystalline carrier and compound or complex formation. Other factors such as increased wettability,  
30 solubilization of the drug by the carrier at the diffusion layer, and the reduction or absence of aggregation and agglomeration may also contribute to increased dissolution.

Drugs having a dissolution-limited oral absorption might benefit from a reduction in particle size, as pointed out in the following equation that is a modification of the well-  
35 known Noyes-Whitney relation:

$$\frac{dM}{dt} = \frac{AD(C_s - C_t)}{h}$$

where  $dm/dt$  is the dissolution rate,  $A$  the specific surface area of the drug particle,  $D$  the diffusion coefficient,  $h$  the diffusion layer thickness,  $C_s$  the saturation solubility, and  $C_t$  the drug concentration at time  $t$ . Since the surface area increases with decreasing particle size, higher dissolution rates may be achieved through reduction of the particle size of the formulations. This effect has been highlighted by the superior dissolution rates after micronisation of certain sparingly water soluble drugs as opposed to regularly milled forms. However, particle size reduction does not necessarily always result in the expected improvement in dissolution rate. This effect arises as a result of the decrease of the effective surface area due to agglomeration and aggregation of very fine particles due to the increased surface energy and subsequent stronger van der Waals' attraction between non-polar molecules. Therefore, the surface of the particles needs to be protected from agglomeration and lyophilic colloids can serve for this purpose, the result being a polymer matrix in which the hydrophobic drug particles are homogeneously dispersed. The controlled chemical passivation (stabilisation) of extremely active (metal) particles of colloidal size using high-molecular-weight compounds was previously described by Pomogailo in 1997.

## SUMMARY OF THE INVENTION

The present invention is based on the unexpected finding that a substantial portion of particles of a bioactive compound suspended in a fluid can be significantly reduced in size by flowing one or more times said fluid having a bioactive compound suspended therein through one or more magnetic fields.

It is a first object of the present invention to reduce the size of solid bioactive compound particles in an aqueous suspension by flowing the suspension through one or more magnetic fields. In a preferred embodiment the particle size of the suspended bioactive compound is reduced from the micrometer to the nanometer range. In a more preferred embodiment the micrometer range refers to particle sizes varying between 1000 and 100,000 nm and the nanometer range refers to particle sizes below 1000 nm, preferably below 500 nm.

It is a second object of the invention to protect the suspended nano-sized bioactive compound particles from agglomeration by mixing the suspension comprising the

bioactive compound particles with stabilising agents, such as protective colloids, surfactants or polymers or a combination thereof. Said stabilising agents can be added before, during or after flowing the suspension through said magnetic field. In case polymers are used the polymers can be selected out of the non-limiting list of  
5 cellulose derivatives (e.g. hydroxy propyl cellulose methyl cellulose, or hydroxy propyl methyl cellulose), polyvinylpyrrolidon, polyvinylpyrrolidone-co-vinylacetate, polyethyleneglycol, ... In case surfactants are used the surfactant can be selected from the non-limiting list of.....

If necessary, the stabilised nanoparticles can be recovered from the suspension by  
10 freeze-drying. In a preferred embodiment the suspension comprising the nano-sized bioactive compound particles was frozen immediately after the preparation of the mixture. Thereafter, the frozen mixture was freeze dried in order to obtain a solid dispersion.

15 A third object of the present invention is a method for the preparation of a solid dispersion containing a bioactive compound comprising the following steps:

1. Preparing a suspension comprising particles of bioactive compounds and one or more stabilising agents
2. flowing said suspension through one or more magnetic fields
- 20 3. Instantaneous freezing of this mixture
4. Freeze drying of the preparation to obtain a solid dispersion

A fourth object of the present invention is a solid dispersion obtainable by said method, containing homogeneously dispersed particles of a bioactive compound,  
25 said particles having a particle size within the nanometer range.

It is well known that drugs having a dissolution-limited oral absorption benefit from a reduction in particle size. Therefore, a fifth object of the present invention is the use of the indicated solid dispersion for the oral administration of a drug, having a low  
30 solubility and dissolution rate, to an animal or human being in need thereof. Typically the drugs having a dissolution-limited oral absorption are classified as a class II or class IV compounds in the Biopharmaceutics Classification System. Further, it should be noted that among the more recently developed bioactive compounds and drugs, proteins and peptides represent a major part. These compounds are often poorly  
35 permeable, poorly soluble, unstable in physiological fluids, with rapid drug metabolism *in vivo* and unfavourable pharmacokinetics. So it is clear that the solid

dispersion of the present invention may also prove useful for the delivery of protein and peptide drugs.

## 5 LIST OF FIGURES

Figure 1: A. Closed system used for the magnetic treatment of the suspension. B. Closed system without an internal magnet (used for blank treatment).

Figure 2: Particle size distribution of Tween micelles in water.

10 Figure 3: Particle size distribution of two magnetically treated diazepam samples compared to an untreated reference sample.

Figure 4: Particle size distribution of a magnetically treated diazepam sample compared to an untreated reference sample after filtration with 1  $\mu$ m pores.

Figure 5: Dissolution profiles of itraconazole from solid dispersion with HPMC.

15 Figure 6: Dissolution profiles of itraconazole from solid dispersion with HPMC. The solid dispersions REC1.1 and REC1.2 were prepared using amorphous itraconazole, while the solid dispersion REC1.3 and REC1.4 were prepared using crystalline itraconazole.

Figure 7: XRD-diffractograms of untreated Itraconazole

20 Figure 8: XRD-diffractograms of a solid dispersion: Itraconazole + HPMC magnetically treated 2820 passes

Figure 9: XRD-diffractograms of a solid dispersion: Itraconazole + tween blanc recirculated 3525 passes

Figure 10: XRD-diffractograms of a solid dispersion: Itraconazole + tween magnetically treated 2820 passes

25 Figures 11 to 15: Electron microscopic images of a magnetically treated solid dispersion.

Figures 16 to 17: Electron microscopic images of a blank treated solid dispersion.

## DETAILED DESCRIPTION OF THE INVENTION

30 The invention relates to the reduction of the size of solid drug particles in aqueous suspensions by flowing the suspensions through magnetic fields. The particle size is reduced from the micrometer to the nanometer range. The drug nanoparticles, which tend to be unstable, can be stabilised and protected from agglomeration with protective colloids, surfactants or polymers or a combination thereof. To recover the  
35 stabilised nanoparticles from the suspension the suspension is preferably frozen and subsequently freeze-dried. In this way a solid dispersion, in which the hydrophobic

drug particles are homogeneously dispersed, can be obtained. In comparison to the current procedures for the preparation of solid dispersions, the present invention clearly offers the advantage that an all-aqueous system is used, thus avoiding organic solvents. A second advantage is that no heat is required to reduce the particle size or to disperse the drug into the protective carrier, which offers the possibility to formulate thermally unstable drugs in solid dispersions.

In our experiments, two poorly soluble drugs were used as model compounds: itraconazole and diazepam. Itraconazole is a potent antifungal drug of the triazole group with activity against histoplasmosis, blastomycosis and onchomycosis. The pharmacological mechanism is the same as that of the structural analogues ketoconazole and miconazole, which interfere with the synthesis of ergosterol of the fungal membrane by inhibition of 14  $\alpha$ -demethylase, a CYP 450 iso-enzyme (Barone et al, 1993). Because of its very low aqueous solubility ( $S < 1 \mu\text{g/ml}$ ) and poor dissolution rate, itraconazole shows a large inter-individual difference in bioavailability after oral administration (Grant and Clissold, 1989). It is classified as a class II compound in the Biopharmaceutics Classification System.

Diazepam is a member of the 1,4-benzodiazepine group, and is clinically used in the treatment of anxiety, insomnia, and as an adjuvant for anesthesia. Like other members of the 1,4-benzodiazepine group, it shows poor water solubility and dissolution properties. In comparison with itraconazole it is less hydrophobic.

#### **EXAMPLE 1: USE OF MAGNETIC TREATMENT TO REDUCE THE PARTICLE SIZE OF DIAZEPAM**

The experiments presented here clearly demonstrate that the particle size of the diazepam particles in water is reduced after re-circulating the suspension through a magnetic field. DLS (dynamic light scattering) measurements and filtration tests demonstrated the reduction of the particle size. DLS provided an estimate of the dimensions of the dispersed particles, while the filtration tests allowed quantifying the amount of dispersed particles within given size classes.

##### Experiment 1: measurement of Tween micelle size

The efficacy of the method of the present invention to reduce the particle size of diazepam was studied using DLS. However, in all experiments described below Tween 80 (Uniqema, Imperial Chemical Industries PLC, London, UK) was added to



the suspension comprising the diazepam particles and it was expected that part of the added Tween would be present as nanometer sized micelles. In order to confirm this hypothesis the size of Tween 80 micelles in water was determined. The information on the micelle size of Tween in water was later on used in the interpretation of the DLS measurements on the suspensions comprising diazepam.

#### Material and Methods

48 mg of Tween 80 was mixed with 20 ml of bi-distilled water and stirred for a few minutes at 100 rpm on a magnetic stirrer.

#### Results

Figure 2 shows that Tween 80 organises itself in water into micelles with a mean diameter of about 10 nm.

#### Experiment 2 : Particle size distribution in an untreated and magnetic treated suspensions of diazepam

In this experiment the effect of magnetic treatment on the particle size distribution of suspended diazepam particles was determined using DLS.

#### Material and Methods

##### *Preparation of the suspension*

50 mg of diazepam (alpha Pharma NV, Zwevegem, Belgium) and 48 mg of Tween 80 (Uniqema, Imperial Chemical Industries PLC, London, UK) were crushed in a mortar. 150 ml of bi-distilled water was added and the suspension was sonicated for 20 minutes. After sonication a considerable amount of material still precipitated, therefore, the suspension was continuously stirred with a magnetic stirrer at 600rpm until further use.

##### *Magnetic treatment of the suspension*

Magnetic treatment was performed in a closed system (Fig.1 A) having a total volume of 100 ml and consisting of (1) a tubing (Masterflex Tygon lab I/P 70, Cole-Parmer Instrument Company, Illinois, USA), (2) an internal magnet of the Al-Ni-Co type, (W, SAN R1/4D, CEPI-CO, Borgerhout, Belgium), (3) a 3-way horizontal ball

valve (Georg Fischer Rohrleitungssysteme AG, type 343 DN10/15, Schaffhausen, Switzerland) and (4) a pump (Masterflex I/P, Cole-Parmer Instrument Company, Illinois, USA). After introducing the diazepam suspension in the closed system 10 to 15 ml of air remained in the system. Upon activation of the pump the suspension was re-circulated through the magnetic field.

After 30 and 60 minutes of treatment, corresponding to 1410 and 2820 passes through the magnetic field, respectively, samples were taken. Immediately after sampling DLS measurements were performed using a He-Ne laser high performance particle sizer (2.5mW) commercially available from ALV (Germany). The samples were shaken before placing them in the particle sizer.

After 80 minutes of treatment corresponding to 3760 passes a third sample was taken. This sample was filtered over a Puradisc 25 AS disposable filter with a polysulfone membrane (Whatman International LTD., Maidstone, England) having a pore size of 1  $\mu\text{m}$ . Thereafter, DLS measurements were performed on the filtrate.

These measurements were compared to those on the filtrate of the untreated suspension.

#### Results and discussion

Figure 3 shows that both magnetically treated samples have significant amounts of particles smaller than 1  $\mu\text{m}$ , with maximums at 11-13 nm and 200-250 nm. The observations at approximately 10 nm are very likely related to the presence of Tween micelles (see experiment 1). The untreated reference sample contains very few particles below 1  $\mu\text{m}$  and a maximum (in number of counts for the suspended particles) is seen at 2,5  $\mu\text{m}$ .

In the filtrate of the untreated suspension two maximums were seen at 11 nm (Tween micelles) and at 200 nm, respectively (Figure 4). On the other hand, the particle size distribution in the filtrate of the magnetically treated suspension was comparable to that of the unfiltered magnetically treated samples shown in Figure 3.

The analysis of the particle distribution in the filtrate of the untreated suspension shows that the untreated sample comprises Tween micelles (peak at 11nm) and 200 nm diazepam particles (Figure 4). However, these particles were not revealed when analysing the unfiltered, untreated suspension (see fig.3) because their presence was masked by the abundance of particles larger than 1  $\mu\text{m}$ . On the other hand, the presence of particles smaller than 1  $\mu\text{m}$  was evident in the unfiltered magnetically treated suspension, indicating that magnetic treatment reduced the particle size of a

substantial portion of the suspended diazepam below 1  $\mu\text{m}$ . Therefore, it can be assumed that the filtrate of the magnetically treated suspension comprised a higher amount of suspended matter than the filtrate of the untreated suspension. This is supported by the finding that Tween micelles (10 nm) are observed as often as 200 nm diazepam particles in the filtrate of the untreated suspension, while in the filtrate of the magnetically treated suspension the 200 nm diazepam particles are more frequently observed. The higher frequency of observation of diazepam (200 nm) particles in the filtrate of the magnetically treated suspension can be explained by two elements:

1. The particle size reduction due to magnetic treatment resulted in more diazepam particles below 1  $\mu\text{m}$  and thus more diazepam particles in the filtrate
2. The particle size reduction of the suspended diazepam particles due to magnetic treatment resulted in a higher particle surface and thus allowing more Tween to be adsorbed on the diazepam particles.

### Experiment 3 : Filter quantification

While DLS measurements allowed estimating the dimensions of the dispersed particles it does not allow performing a quantitative analysis. Therefore, filtration tests were performed to quantify the amount of diazepam particles in given size classes.

### Material and Methods

#### *Preparation of the suspension*

600 mg of diazepam (producent) and 610 mg of Tween 80 (Uniqema, Imperial Chemical Industries PLC, London, UK) were mixed with bi-distilled water in a mortar. The suspension was poured in a 600 ml beaker and bi-distilled water was added in order to obtain a volume of 300 ml. The suspension was sonicated during 20 minutes. Because a considerable amount of powder still precipitated after sonication, the suspension was continuously stirred (600 rpm) using a magnetic stirrer.

#### *Magnetic treatment of the suspension*

Part of the suspension was subjected to magnetic treatment in a closed system (Fig.1 A) having a total volume of 150 ml and consisting of (1) a tubing (Masterflex Tygon lab I/P 70, Cole-Parmer Instrument Company, Illinois, USA), (2) an internal

magnet of the Al-Ni-Co type, (W, SAN R1/4D, CEPI-CO, Borgerhout, Belgium), (3) a 3-way horizontal ball valve (Georg Fischer Rohrleitungssysteme AG, type 343 DN10/15, Schaffhausen, Switzerland) and (4) a pump (Masterflex I/P, Cole-Parmer Instrument Company, Illinois, USA). After introducing the diazepam suspension in the closed system 10 to 15 ml of air remained in the system. Upon activation of the pump the suspension was re-circulated through the magnetic field. After 90 minutes of treatment, corresponding to 2820 passes through the magnetic field a sample was collected for filtration.

Another part of the initial suspension was continuously stirred with a magnetic stirrer at 600 rpm during 1 hour (untreated reference sample).

#### *Filtration of the samples*

ProFill syringe filters with PTFE and a pore size of 0.45  $\mu\text{m}$  (Alltech Associates Inc., Illinois, USA) were washed with bi-distilled water and dried for more than 12 hours at 70 °C. ProFill syringe filters with PTFE and a pore size of 0.2  $\mu\text{m}$  (Alltech Associates Inc., Illinois, USA) were not washed nor dried. Immediately after treatment 40 ml of the magnetically treated suspension was filtered over the 450 and 200 nm filters. Similarly, 40 ml of the untreated reference suspension was filtered over the 450 and 200 nm filters. The filtrate was poured into petridishes. The petridishes and the filters were dried for approximately 48 hours at 70°C.

#### Results and Discussion

Data regarding the retention of material on the filters are given in table 1.

Table 1: Amount of material retained on the filter as a percentage of the total dry mass

sample	filter	% of dry mass retained by filter
untreated reference	450 nm	47
untreated reference	200 nm	47
magnetically treated	450 nm	<1
magnetically treated	200 nm	1

Assuming that no surfactant (Tween 80) is retained during filtration it can be concluded that almost all diazepam (94%) is retained by both the 450 nm and 200 nm filters in the untreated reference sample. In the magnetically treated sample on

the other hand approximately all of the diazepam appears to be smaller than 200 nm and is therefore not retained by any of the filters.

#### Experiment 4 : filter quantification

5

Different diazepam suspension were prepared and for each suspension the amounts of diazepam particles in given size classes was quantified.

#### Material and Methods

10

##### *Preparation of the suspension*

800 mg of diazepam (producent) and 800 mg of Tween 80 (Uniqema, Imperial Chemical Industries PLC, London, UK) were mixed with bi-distilled water in a mortar. The suspension was poured in a 600 ml beaker and bi-distilled water was added in order to obtain a volume of 400 ml. The suspension was sonicated during 20 minutes. As a considerable amount of powder still precipitated after sonication the suspension was continuously stirred (600 rpm) using a magnetic stirrer.

##### *Magnetic treatment of the suspension*

Part of the suspension was subjected to magnetic treatment in a closed system (Fig.1 A) having a total volume of 150 ml and consisting of (1) a tubing (Masterflex Tygon lab I/P 70, Cole-Parmer Instrument Company, Illinois, USA), (2) an internal magnet of the Al-Ni-Co type, (W, SAN R1/4D, CEPI-CO, Borgerhout, Belgium), (3) a 3-way horizontal ball valve (Georg Fischer Rohrleitungssysteme AG, type 343 DN10/15, Schaffhausen, Switzerland) and (4) a pump (Masterflex I/P, Cole-Parmer Instrument Company, Illinois, USA). After introducing the diazepam suspension in the closed system 10 to 15 ml of air remained in the system. Upon activation of the pump the suspension was re-circulated through the magnetic field. After 90 minutes of treatment, corresponding to 2820 passes through the magnetic field a sample was collected for filtration.

30

A second part of the suspension was treated in a 150 ml closed system lacking the magnetic device (Figure 1B). After 90 minutes of treatment, corresponding to 2820 passes a sample was collected for filtration.

A third part of the initial suspension was continuously stirred with a magnetic stirrer at 600 rpm for at least 1 hour (untreated reference sample).

35

### *Filtration of the samples*

ProFill syringe filters with PTFE and a pore size of 0.45 µm (Alltech Associates Inc., Illinois, USA) were washed with bi-distilled water and dried for more than 12 hours at 70 °C. ProFill syringe filters with PTFE and a pore size of 0.2 µm (Alltech Associates Inc., Illinois, USA) were not washed nor dried. Immediately after treatment 40 ml of the magnetically treated suspension was filtered over the 450 and 200 nm filter. Similarly, 40 ml of the untreated reference suspension was filtered over the 450 and 200 nm filter. The filtrate was poured into petridishes. The petridishes and the filters were dried for approximately 48 hours at 70°C.

### Results and discussion

Data regarding the retention of material on the filters are given in table 1.

Table 2: Amount of material retained on the filter as a percentage of the total dry mass

sample	filter	% of dry mass retained by filter
untreated reference	450 nm	48
untreated reference	200 nm	47
blanc recirculated	450 nm	16
blanc recirculated	200 nm	21
magnetically treated	450 nm	5
magnetically treated	200 nm	8

Assuming that no surfactant (Tween 80) is retained during filtration it can be concluded that almost all diazepam (94-96%) is retained by both the 450 nm and 200 nm filters in the untreated reference sample. In the blank re-circulated sample 32 % of diazepam is larger than 450 nm and 41 % is larger than 200 nm. In the magnetically treated sample on the other hand only 10 % of diazepam is larger than 450 nm and 16 % is larger than 200 nm. This clearly illustrates the beneficial effect of magnetic treatment on the particle dimensions. The intermediate character of the results of the blank re-circulated sample may be explained by a number of factors comprising increased solubility (heat generated by the pump), abrasion of particles in contact with the closed system, ...

## EXAMPLE 2: DISSOLUTION PROFILES OF ITRACONAZOLE FROM SOLID DISPERSIONS WITH HPMC

- 5 Three solid dispersions of itraconazole/HPMC were prepared using magnetic induction. Thereafter, the dissolution of itraconazole from the preparation was compared to that from a physical mixture of itraconazole and HPMC prepared without the use of magnetic induction.

10 Experiment 1: Dissolution profiles of crystalline itraconazole from a solid dispersion with HPMC

Material and methods

15 *Preparation of itraconazole-HPMC dispersions*

- 500 mg of itraconazole (kindly provided by Janssen Pharmaceutica NV, Beerse, Belgium) was triturated with 8 drops of polysorbate 80 (= Tween 80) (Uniqema, Imperial Chemical Industries PLC, London, UK) and suspended in 10 ml of water. This suspension was added to a closed circuit containing 790 ml of water and 5  
20 magnets. Itraconazole was circulated by means of a pump for 24 hours at a speed of 4 l/min. After 24 hours, 200ml of the itraconazole suspension was isolated by rapidly pouring it in a solution of HPMC (90mg in 10ml of water), followed by mixing with a rotor stator homogenizer for 30 seconds. This mixture was then immediately poured in an excess of liquid nitrogen. Subsequently, the sample was freeze dried, and the  
25 freeze dried samples were tested for their dissolution behaviour. The ratio of itraconazole to HPMC was 40:60. This preparation is referred to as Formulation 1.

- In the preparation of the second dispersion, 250 mg of itraconazole was used instead of 500mg (Formulation 2).

- In the preparation of the third dispersion, 250 mg of itraconazole was used and the  
30 ratio of itraconazole to HPMC was 20:80 (Formulation 3).

The control preparation consisted of a physical mixture of itraconazole with HPMC.  
(Physical mixture = dry mixed in a mortar)

*Dissolution of itraconazole*

- 35 Dissolution experiments were performed using the USP 24 method (paddle method, 100 rpm). Samples (corresponding to 50 mg of itraconazole) were added to the dissolution medium made up of simulated gastric fluid without pepsin (USP 24)

and the temperature of the dissolution medium was maintained at  $37 \pm 0.1^\circ\text{C}$ . Samples of 2 ml were taken and immediately replaced with fresh dissolution medium at 5, 10, 20, 30, 60, 90, and 120 minutes and then filtered with Teflon filters of  $0.45\mu\text{m}$  (Acrodisc, Pall Corporation, New York, USA) into HPLC vials (1.5 ml, Merck, Darmstadt, Germany). The corresponding concentrations of itraconazole were determined from the calibration curve with HPLC.

*Formula of the dissolution medium*

NaCl	2g
HCl	7g
Purified water	to 1L

*HPLC analysis of itraconazole*

The HPLC system consisted of LiChroGraph® L-7100 HPLC pump, an autosampler model L-7200 equipped with a 100µl loop, a UV detector model L-7400 set at 257 nm, and an Interface D-7000, all from Merck-Hitachi (Darmstadt, Germany). UV signals were monitored and peaks were integrated using the D-7000 HSM software. All chromatographic separations were performed at room temperature. The column used was LiChrospher® 100 RP-18 ( $5\mu\text{m}$ )  $12.5 \times 4$  (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile/tetrabutylammonium hydrogen sulphate 0.01N (50:50; v/v) and was degassed by ultrasonication before use. The flow rate amounted to 1 ml/min. The retention time of itraconazole at these conditions was 11.5 min.

Results

Figure 5 clearly shows that the dissolution of itraconazole from the formulations prepared using magnetic induction was much faster than from the control formulation. It was also observed that the equilibrium concentrations (i.e. the concentration after 120 min) of itraconazole in the dissolution medium obtained with Formulation 1, 2 and 3 were significantly higher than those obtained with the Control Formulation.

Experiment 2: Dissolution profiles of crystalline and amorphous itraconazole from solid dispersion with HPMC



Two different types of itraconazole-containing solid dispersions were prepared using the method of the present invention. The first was prepared by conducting a suspension of crystalline itraconazole through the magnetic field, while for the preparation of the second type a suspension of amorphous itraconazole was used. In both dispersions hydroxypropyl methylcellulose (HPMC 2910) was used as stabilising polymer. The dissolution of itraconazole from the types of solid dispersions was compared.

#### Materials and methods

##### *Preparation of glassy itraconazole*

Glassy itraconazole was prepared by melting crystalline itraconazole followed by rapid cooling to room temperature after which it was milled and sieved ( $<355\mu\text{m}$ ). Glassy itraconazole was stored in a dessicator at room temperature until further analysis (within 1 week).

##### *Preparation and magnetic treatment of the itraconazole-containing suspension.*

A HPMC solution was prepared by dissolving 225 mg of HPMC 2910 in 50 ml of water. Thereafter, a concentrated itraconazole suspension was prepared by mixing 150 mg of itraconazole with 4 drops of Tween 80 and subsequently adding a limited amount of water. This suspension was prepared with crystalline and glassy itraconazole, respectively.

Both the HPMC solution and the concentrated itraconazole suspension were poured into a closed system consisting of tubing (Masterflex Tygon lab I/P 70, Cole-Parmer Instrument Company, Illinois, USA), a commercial AlNiCo magnet (W, SAN R1/4D, CEPI-CO, Borgerhout, Belgium) and a 3-way horizontal ball valve (Georg Fischer Rohrleitungssysteme AG, type 343 DN10/15, Schaffhausen, Switzerland). The tubing was attached to the pump (Masterflex I/P, Cole-Parmer Instrument Company, Illinois, USA). The total volume of this closed system was approximately 0,100 l (measurement conducted with tap water). After the HPMC solution and itraconazole suspension were poured into the closed system approximately 10 ml of air remained in the system.

When conducting the suspension comprising glassy itraconazole through the magnetic field, the suspension was circulated at a pumping speed of 4,7 l/min (measurement conducted with tap water in an open system resembling real experimental conditions). When conducting the suspension comprising crystalline

itraconazole through the magnetic field, the suspension was circulated at a pumping speed of 6,3 l/min. Treatment was continued for 1 hour in both cases, equalling 2820 passes in the case of glassy itraconazole and 3780 passes in the case of crystalline itraconazole. It was observed that the temperature of liquids in the closed system increased to 40-45 °C during treatment.

*Preparation of a solid dispersion by freeze-drying the magnetically treated itraconazole suspension*

Following magnetic treatment, the itraconazole suspension was snap frozen by pumping it into a 1 litre round bottomed flask, which was previously cooled in liquid nitrogen. The frozen sample was lyophilized for about 12 hours in order to obtain a powdered solid dispersion.

*Analysis of the dissolution characteristics of the respective solid dispersions*

The dissolution of itraconazole from the different solid dispersions was evaluated as described above (see Example 2, experiment 1).

Results

Two independent preparations with glassy (REC1.1 & REC1.2) and crystalline itraconazole (REC1.3 & REC1.4), respectively, were prepared. Comparison of the dissolution characteristics of the four preparations showed that much higher dissolution rates were obtained with glassy than with crystalline itraconazole (fig. 6). Two hours after the start of the dissolution test about 50% of the itraconazole was released from the REC1.1 and REC1.2 samples, while about 15% of the itraconazole was released from REC1.3 and REC1.4.

**EXAMPLE 3: X-RAY DIFFRACTION ANALYSIS OF ITRACONAZOLE-CONTAINING SOLID DISPERSIONS**

The presence of nano-particles in the magnetically treated, freeze-dried itraconazole preparations was investigated using X-ray diffraction (XRD). The occurrence of peak broadening in XRD diffractograms is considered to be a good indication that nano-sized particles are present. The critical size causing peak broadening can be estimated. Assuming that (1) size of an itraconazole molecule is about 1.5nm and that (2) the estimated maximal amount of repetitive units causing peak broadening is

about 50, then it can be calculated that only particles smaller than 75 nm contribute to peak broadening.

## Materials and Methods

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### *Preparation of the samples*

1. Untreated Itraconazole: crystalline Itraconazole, as received from Janssen Pharmaceutica NV, not treated in any way whatsoever, without addition of polymer or surfactant.
- 10 2. Itraconazole + HPMC magnetically treated 2820 passes: 450 mg of HPMC 2810 was dissolved in 20 ml of bi-distilled water, while 300 mg of Itraconazole + 6 drops of Tween 80 (+/- 120 mg) were suspended in an amount of bi-distilled water. The HPMC solution and the Itraconazole dispersion were added to the tubing of a closed system with a magnetic field and the internal volume of the  
15 system (100 ml) was filled with bi-distilled water. The suspension was magnetically treated during 1 hour. Immediately thereafter, the magnetically treated suspension was frozen in liquid nitrogen and lyophilised.
3. Itraconazole + tween blanc recirculated 3525 passes: 250 mg of itraconazole was suspended in an amount of bi-distilled water comprising 120 mg of Tween 80.  
20 This suspension was poured into the tubing of a closed system lacking a magnetic field and the remaining volume of the system (80 ml) was filled with bi-distilled water, leaving about 10 ml of air. The dispersion was re-circulated in the absence of a magnetic device during 1 hour. Immediately thereafter, the magnetically treated suspension was frozen in liquid nitrogen and lyophilised.
- 25 4. Itraconazole + tween magnetically treated 2820 passes: 250 mg of itraconazole was suspended in an amount of bi-distilled water comprising 100 mg of Tween 80. The dispersion is poured into the tubing of a closed system comprising an internal magnet and the remaining volume in the system (100 ml) was filled with bi-distilled water, leaving about 10 ml of air. The dispersion was magnetically  
30 treated during 1 hour. The dispersion was re-circulated in the absence of a magnetic device during 1 hour. Immediately thereafter, the magnetically treated suspension was frozen in liquid nitrogen and lyophilised.

### *XRD-analysis*

- 35 The XRD analysis was performed using a Siemens D5000 matic X-ray diffractometer, using a program, which measured from  $2\theta = 0.7$  to  $2\theta = 40$  in steps of 0.02 / 4 seconds.

## Results

The peak widths were calculated for 2 different peaks:

5

### Peak 1 :

Peak width 'itraconazole untreated' = 0.19 (maximum at  $2\theta$  = 20.46) (Figure 7)

Peak width 'itraconazole + HPMC magnetically treated 2820 passes' = 0.27  
(maximum at  $2\theta$  = 20.58) (Figure 8)

10 Peak width 'itraconazole + tween blank treatment 3525 passes' = 0.16 (maximum at  
 $2\theta$  = 20.58) (Figure 9)

Peak width 'itraconazole + tween magnetically treated 2820 passes' = 0.28  
(maximum at  $2\theta$  = 20.7) (Figure 10)

15 **Peak 2 :**

Peak width 'itraconazole untreated' = 0.22 (maximum at  $2\theta$  = 23.58) (Figure 7)

Peak width 'itraconazole + HPMC magnetically treated 2820 passes' = 0.27  
(maximum at  $2\theta$  = 23.68) (Figure 8)

20 Peak width 'itraconazole + tween blank treatment 3525 passes' = 0.16 (maximum at  
 $2\theta$  = 23.70) (Figure 9)

Peak width 'itraconazole + tween magnetically treated 2820 passes' = 0.36  
(maximum at  $2\theta$  = 23.52) (Figure 10)

It is clear that the peak widths observed for the magnetically treated samples were  
clearly higher than in the untreated or blank treated sample indicating that the  
25 magnetically treated samples comprise relatively more nano-sized itraconazole  
particles.

### EXAMPLE 3: ELECTRON MICROSCOPIC ANALYSIS OF ITRACONAZOLE- CONTAINING SOLID DISPERSIONS

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The presence of small particles in the magnetically treated samples was visualised  
using an electron microscope (eSEM).

## Materials and Methods

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### *Preparation of the samples*

1. Itraconazole + tween blank recirculated 3525 passes: 250 mg of itraconazole was  
suspended in an amount of bi-distilled water comprising 120 mg of Tween 80.

This suspension was poured into the tubing of a closed system lacking a magnetic field and the remaining volume of the system (80 ml) was filled with bi-distilled water, leaving about 10 ml of air. The dispersion was re-circulated in the absence of a magnetic device during 1 hour. Immediately thereafter, the magnetically treated suspension was frozen in liquid nitrogen and lyophilised.

2. Itraconazole + tween magnetically treated 2820 passes: 250 mg of itraconazole was suspended in an amount of bi-distilled water comprising 100 mg of Tween 80. The dispersion is poured into the tubing of a closed system comprising an internal magnet and the remaining volume in the system (100 ml) was filled with bi-distilled water, leaving about 10 ml of air. The dispersion was magnetically treated during 1 hour. The dispersion was re-circulated in the absence of a magnetic device during 1 hour. Immediately thereafter, the magnetically treated suspension was frozen in liquid nitrogen and lyophilised.

## Results and Discussion

Clear differences between eSEM pictures of the magnetically treated sample (Fig 11-15) and of the blank re-circulated sample (Fig 16-17) are shown. The greater part of particles of the blank re-circulated sample has at least 1 dimension of a few up to several tens of micrometers. A small amount of particles with sizes of approximately 1  $\mu\text{m}$  is also observed. The itraconazole crystals measure tens of microns and have an euhedral crystal morphology. The magnetically treated sample contains a significant amount of particles with sizes of around 1 micrometer beside a few large residual particles. The clear size difference between the magnetically treated sample and the reference confirm the occurrence of particle size reduction upon magnetic treatment. The micron size particles obtained after magnetic treatment have an irregular morphology. Most likely they are composed of even smaller entities. Unfortunately, focussing on these particles with the eSEM instrument results in melting, as is illustrated in Figure 13, where globular micron size particles are observed, representing drops of molten particles. The fine structure of the particles with diameters of around the micrometer could not be revealed with eSEM.

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**CLAIMS**

1. A method for reducing the average size of bioactive compound particles or agglomerates suspended in a fluid by flowing one or more times said fluid having  
5 bioactive compound particles or agglomerates suspended therein through one or more magnetic fields to reduce the average size of a substantial portion of the bioactive compound particles or agglomerates.
2. The method according to claim 1, wherein the strength of each said magnetic field is at least about 2,000 gauss.
- 10 3. The method according to claim 1 or 2 wherein the fluid is a liquid.
4. The method according to claim 3 wherein the liquid is water.
5. The method according to claims 3 or 4 wherein a stabilizing agent is added to the liquid comprising the bioactive compound particles or agglomerates.
6. The method according to claims 3 or 4 wherein the stabilizing agent is a  
15 surfactant
7. The method according to claims 3 or 4 wherein the stabilizing agent is a polymer
8. The method according to claims 3 or 4 wherein the stabilizing agent is a combination of a polymer and a surfactant.
9. A method for the preparation of a solid dispersion of a bioactive compound  
20 comprising the steps:
  - (i) preparing a suspension of bioactive compound particles according to the method of claims 4 to 8
  - (ii) freezing said suspension
  - (iii) freeze-drying the preparation to obtain a solid dispersion.

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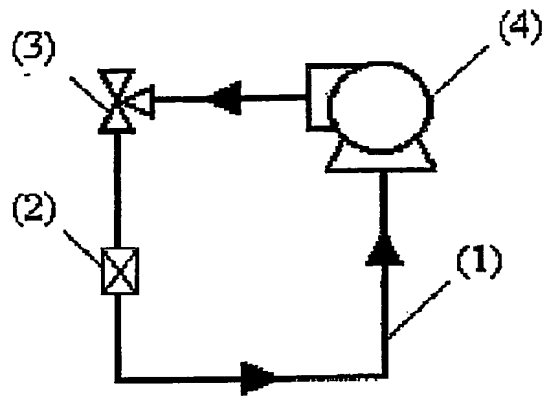
**ABSTRACT**

The invention relates to the reduction of the size of solid drug particles in aqueous suspensions by conduction of the suspensions through magnetic fields. The particle  
5 size is reduced from the micrometer to the nanometer range. Furthermore, the invention relates to methods allowing the stabilisation of the obtained nano-particles as well as to formulations containing said stabilised nano-particles





A



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B

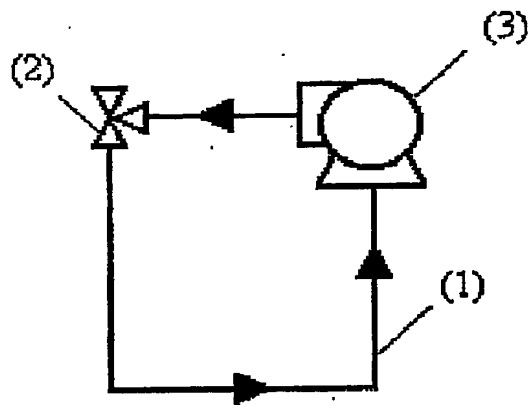


Figure 1

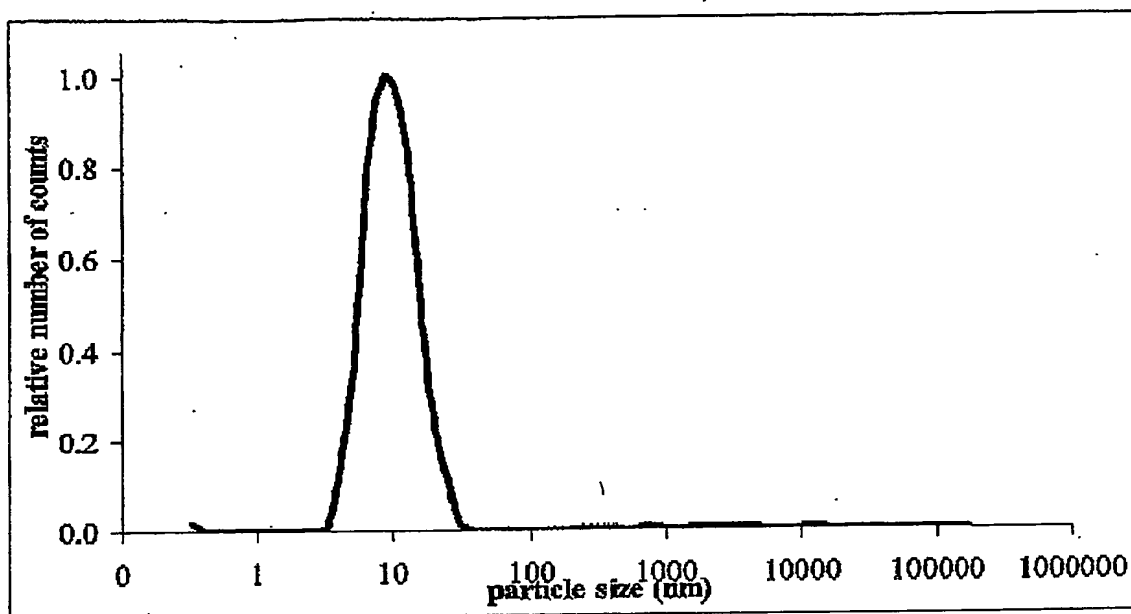


Figure 2

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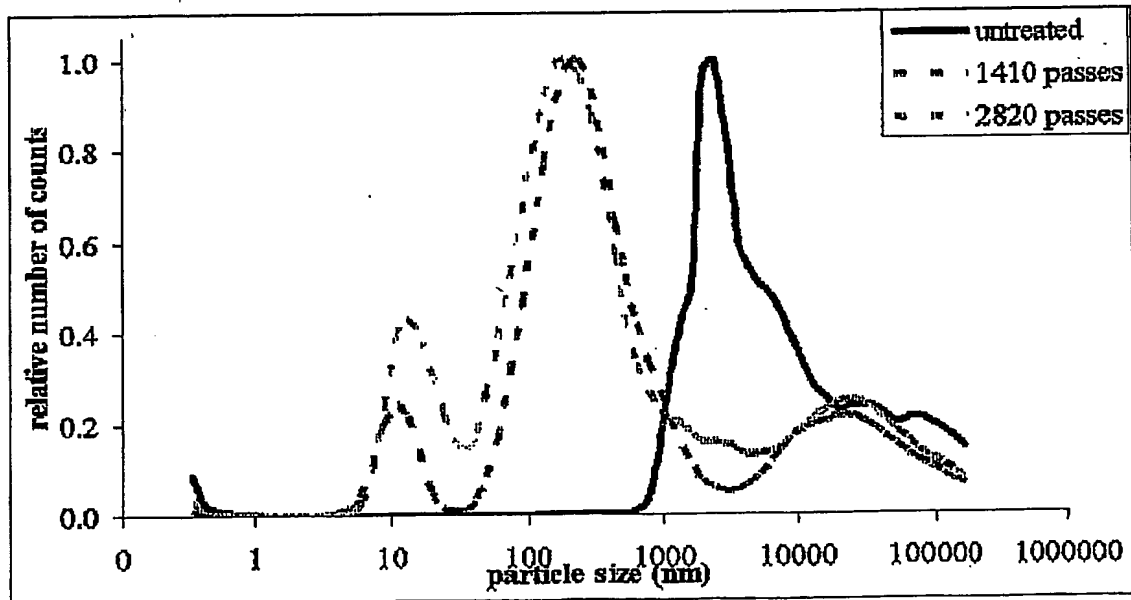


Figure 3

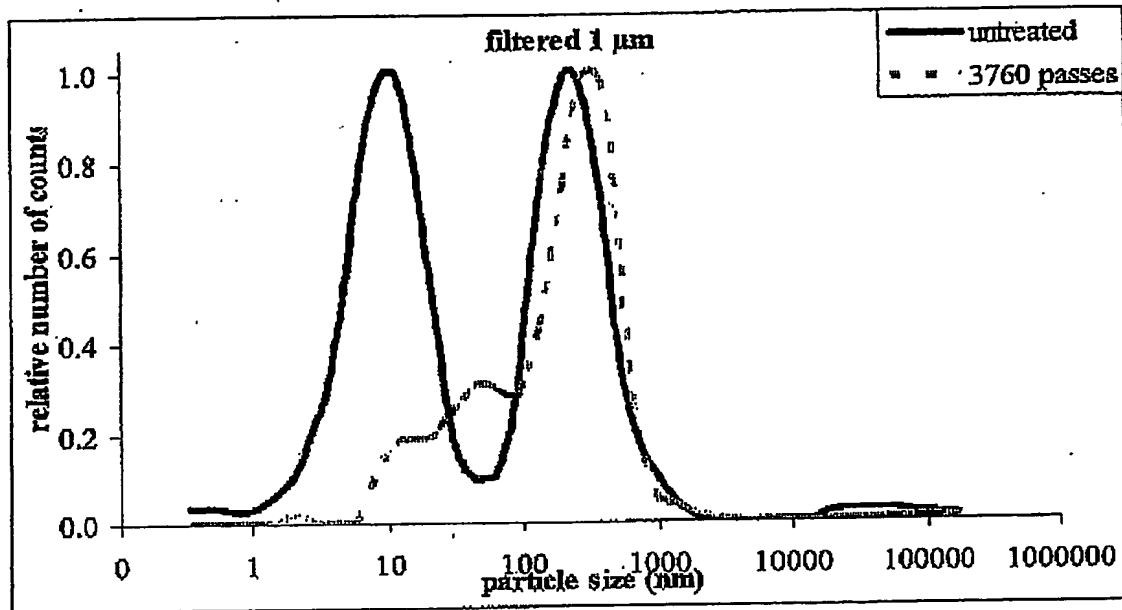


Figure 4

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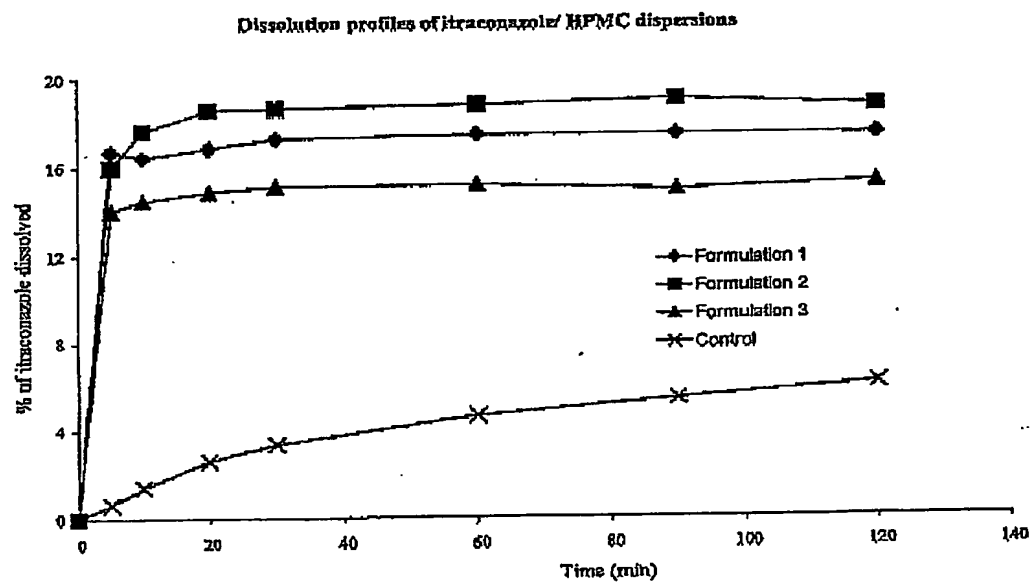


Figure 5

## Dissolution profiles of amorphous and crystalline itraconazole

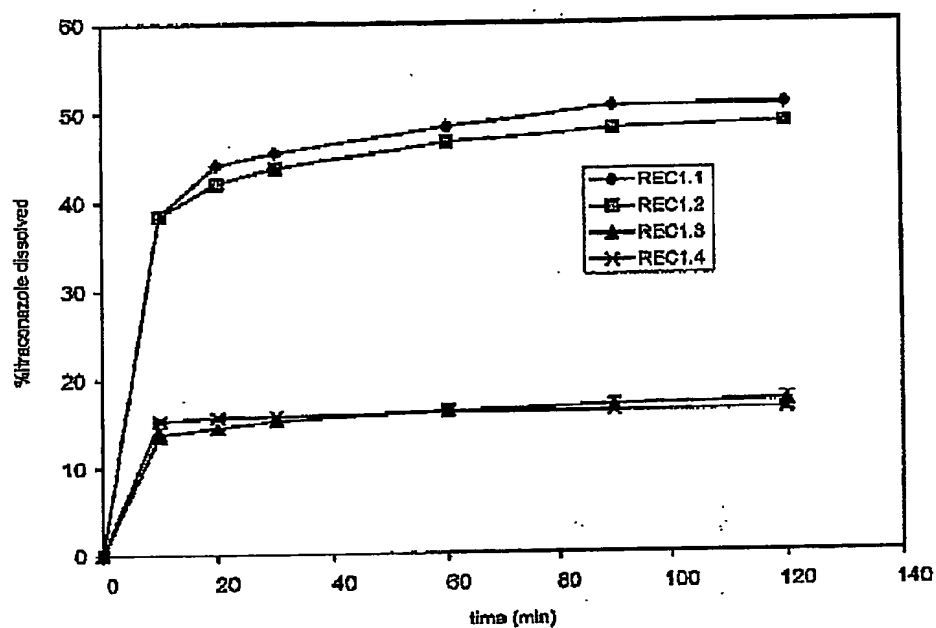
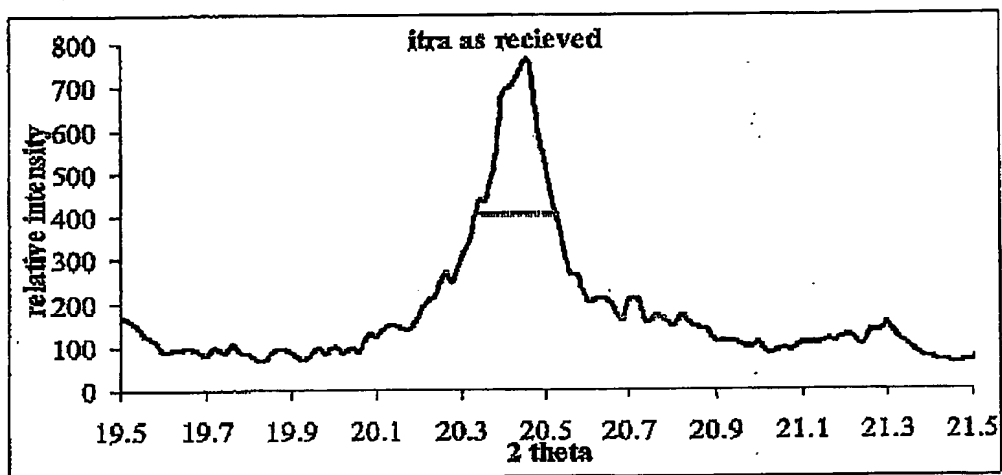
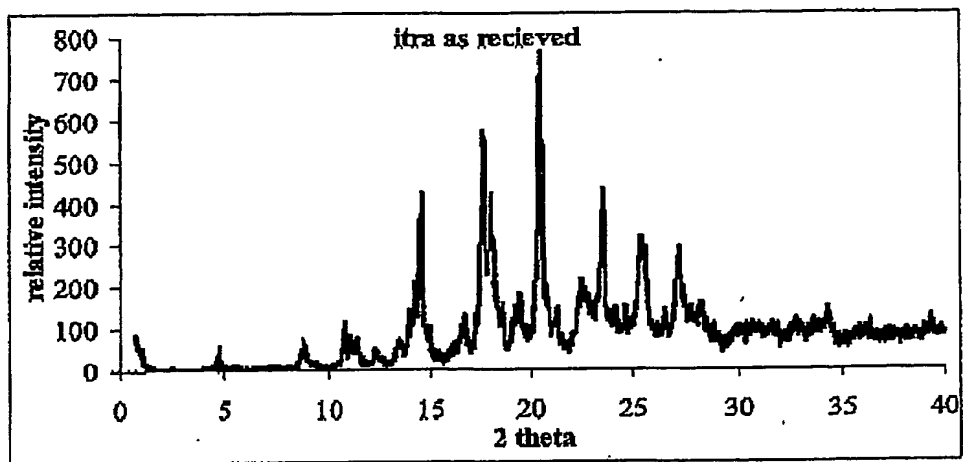


Figure 6



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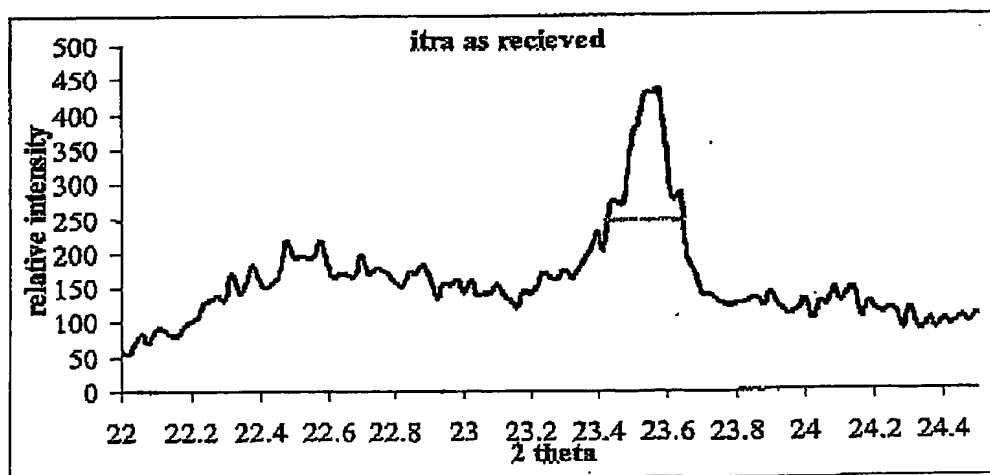


Figure 7

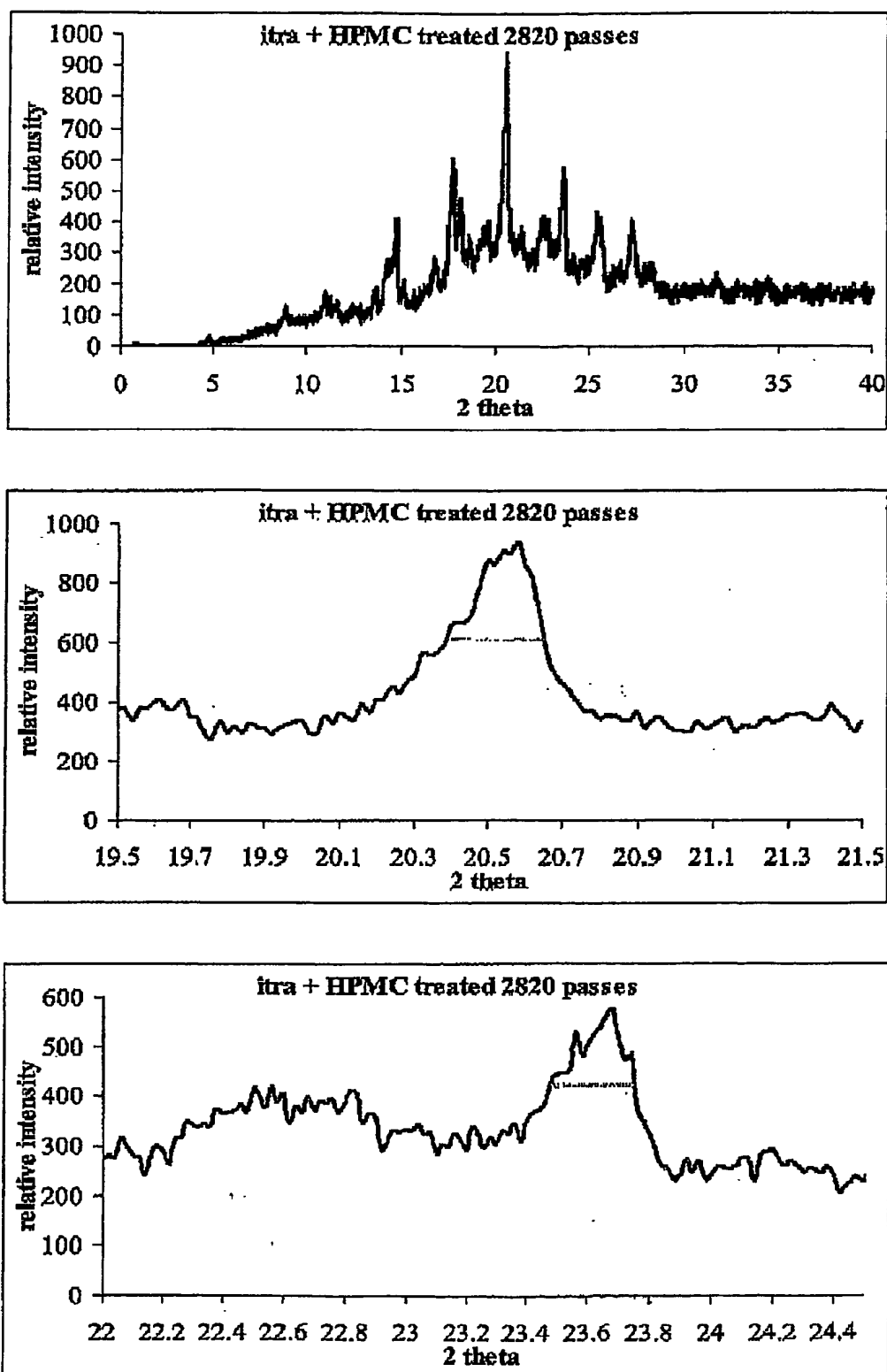


Figure 8

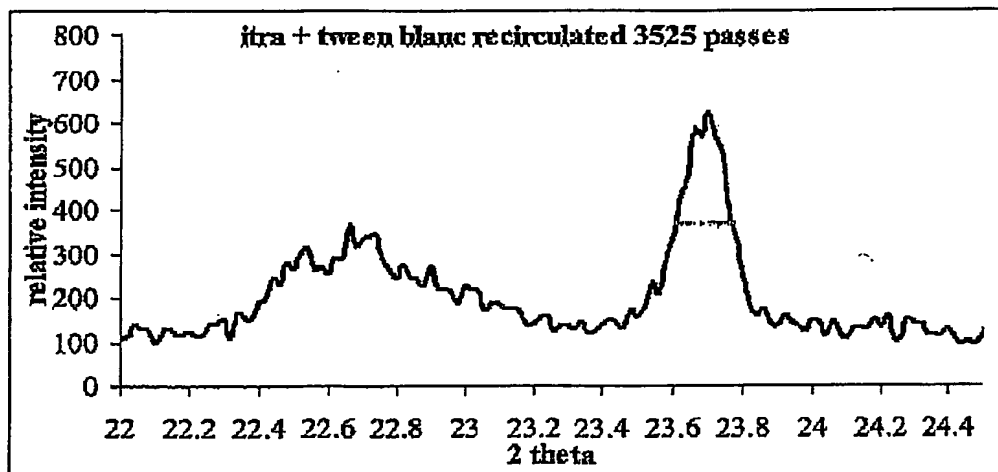
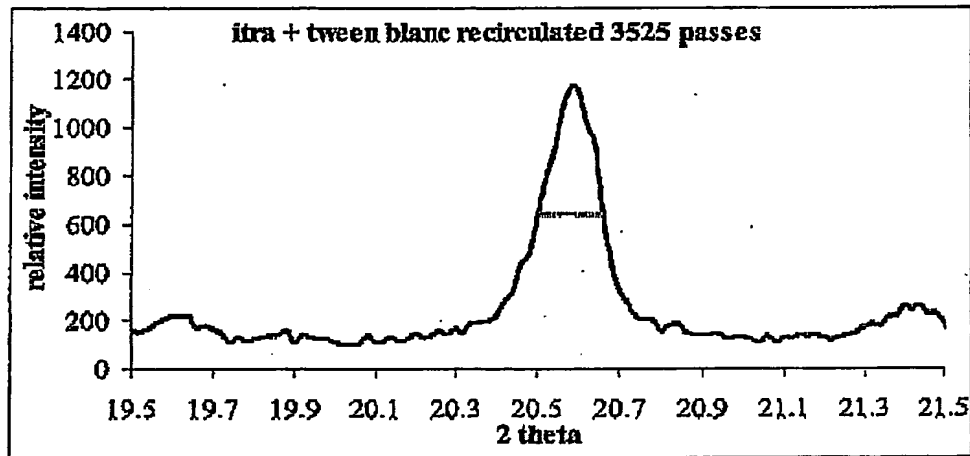
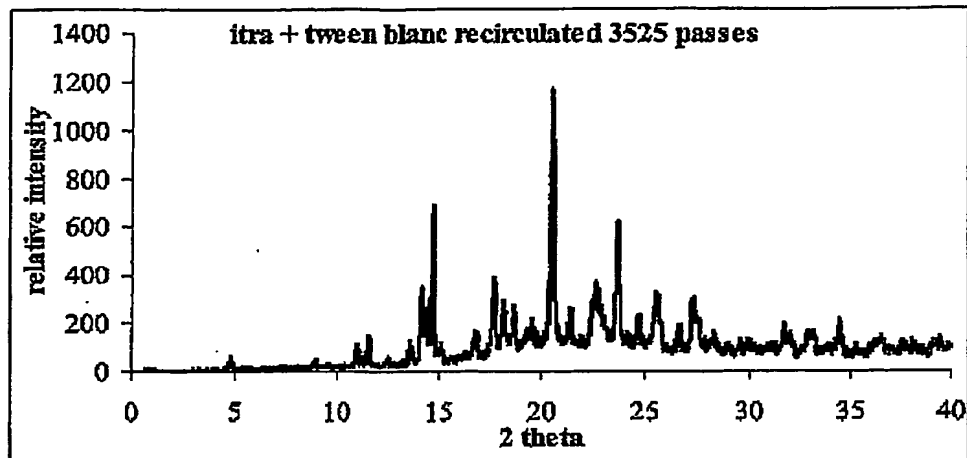
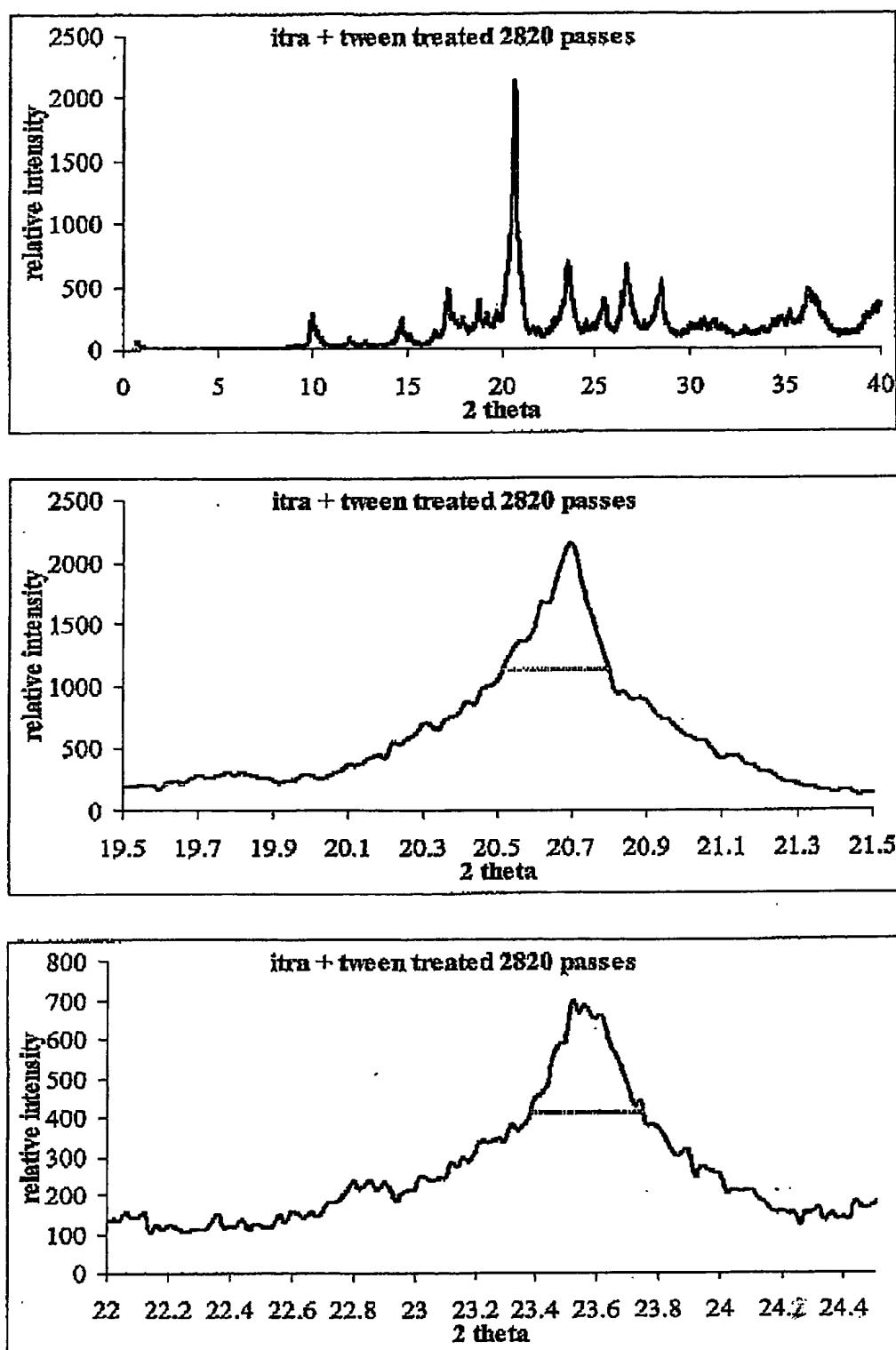


Figure 9



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Figure 10



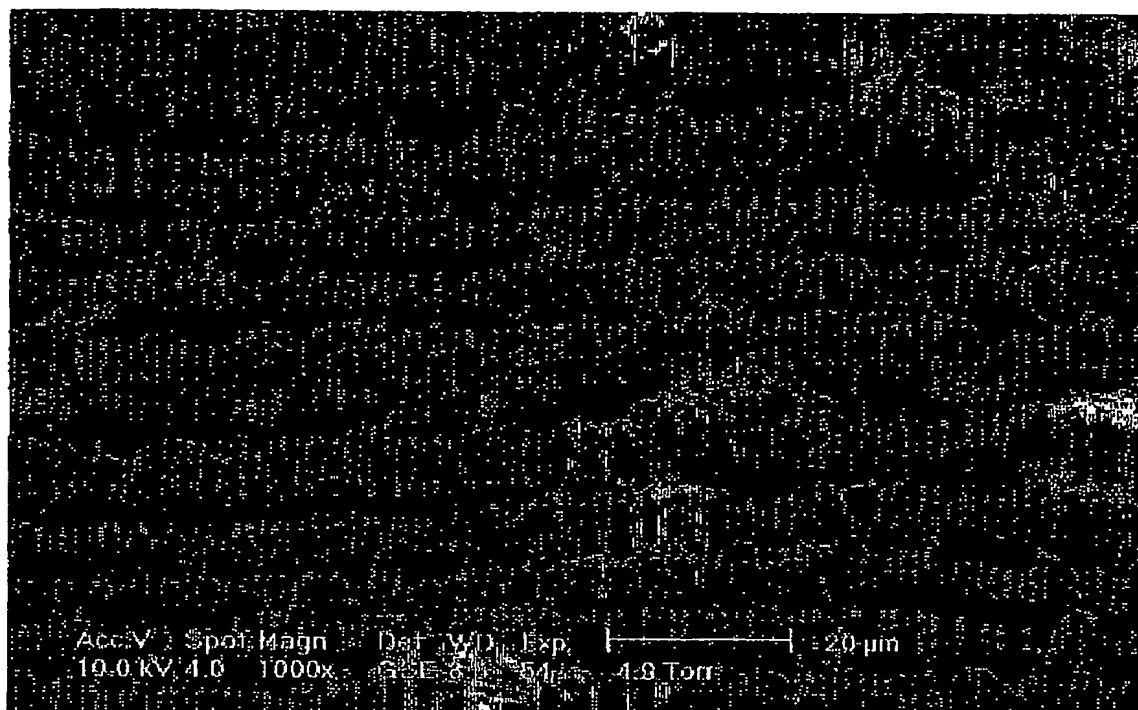


Fig 11

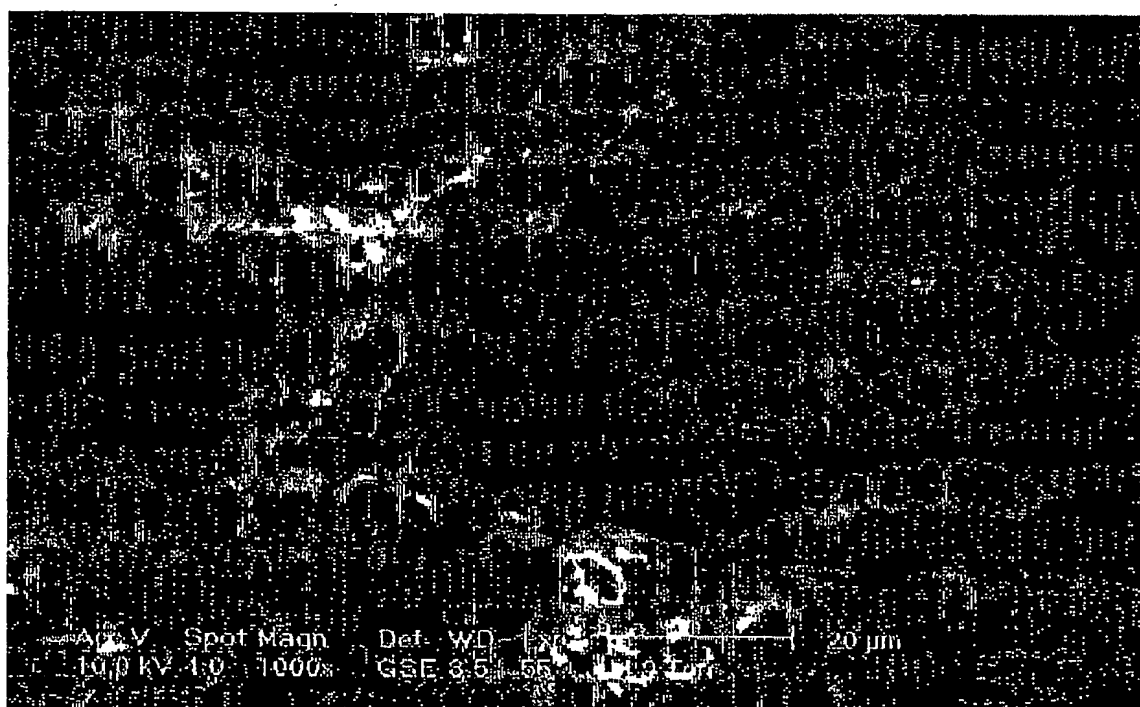


Fig 12

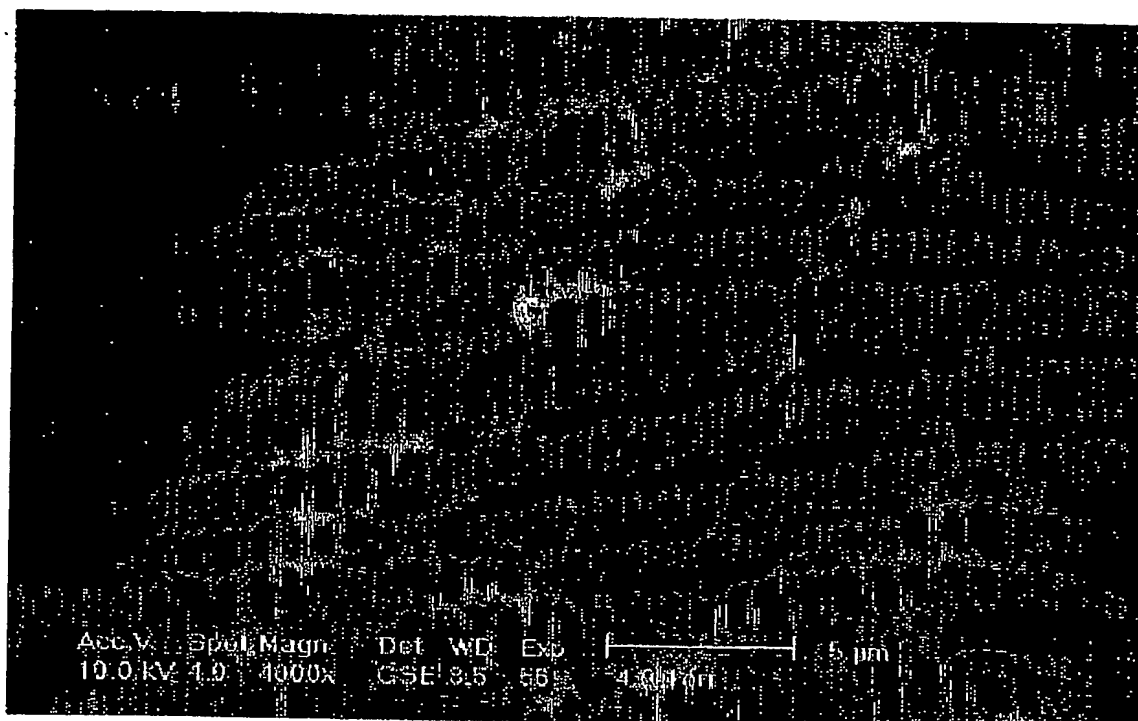


Fig 13

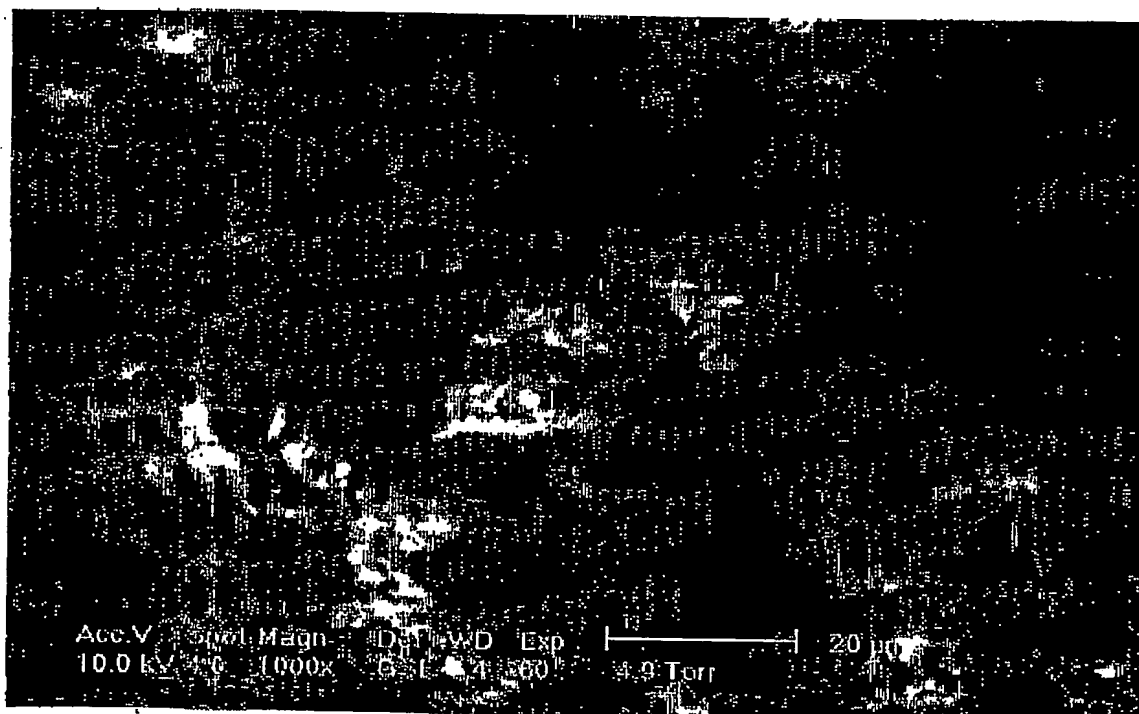


Fig 14

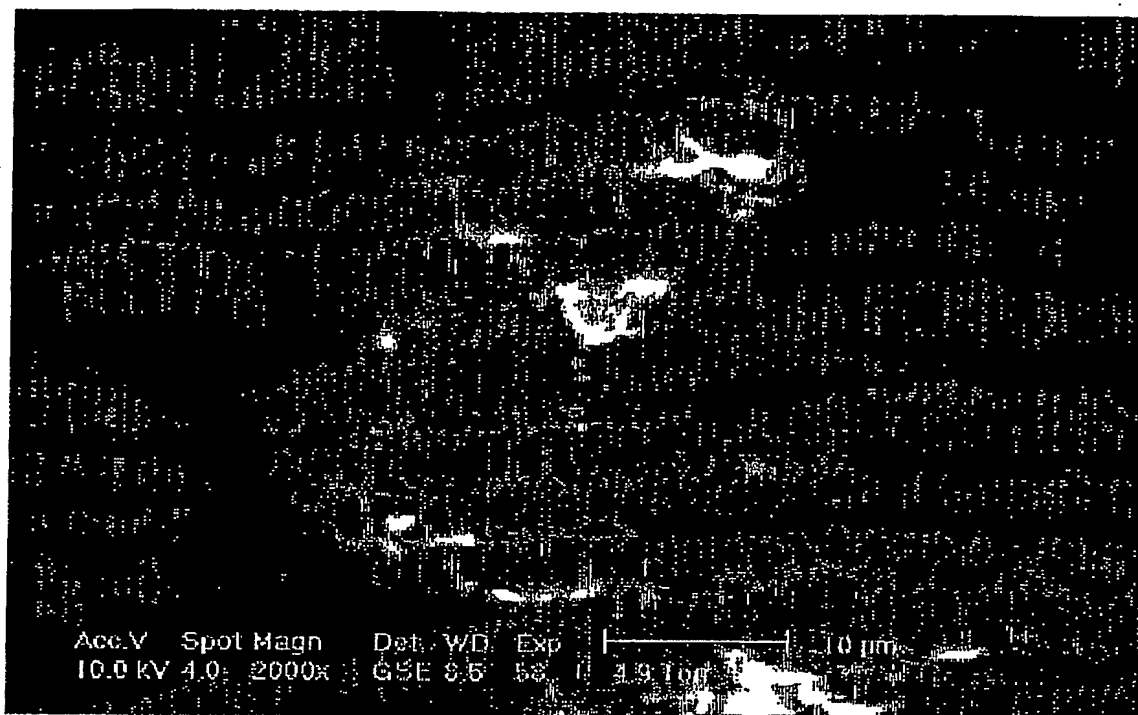


Fig 15

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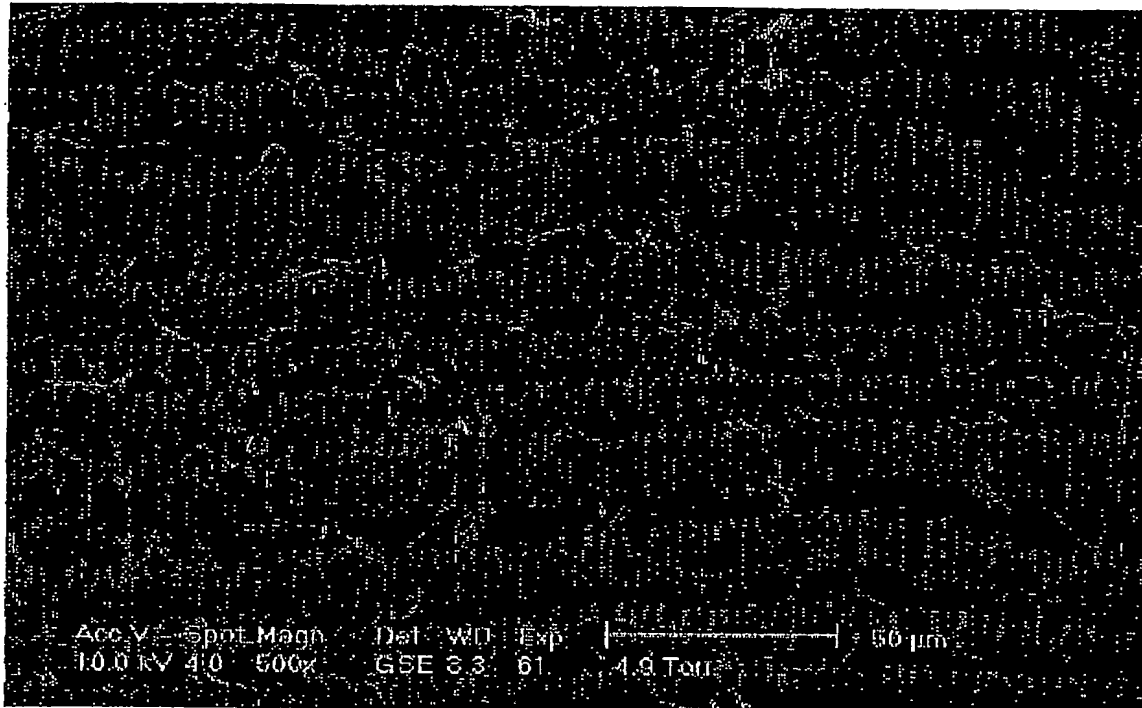


Fig 16

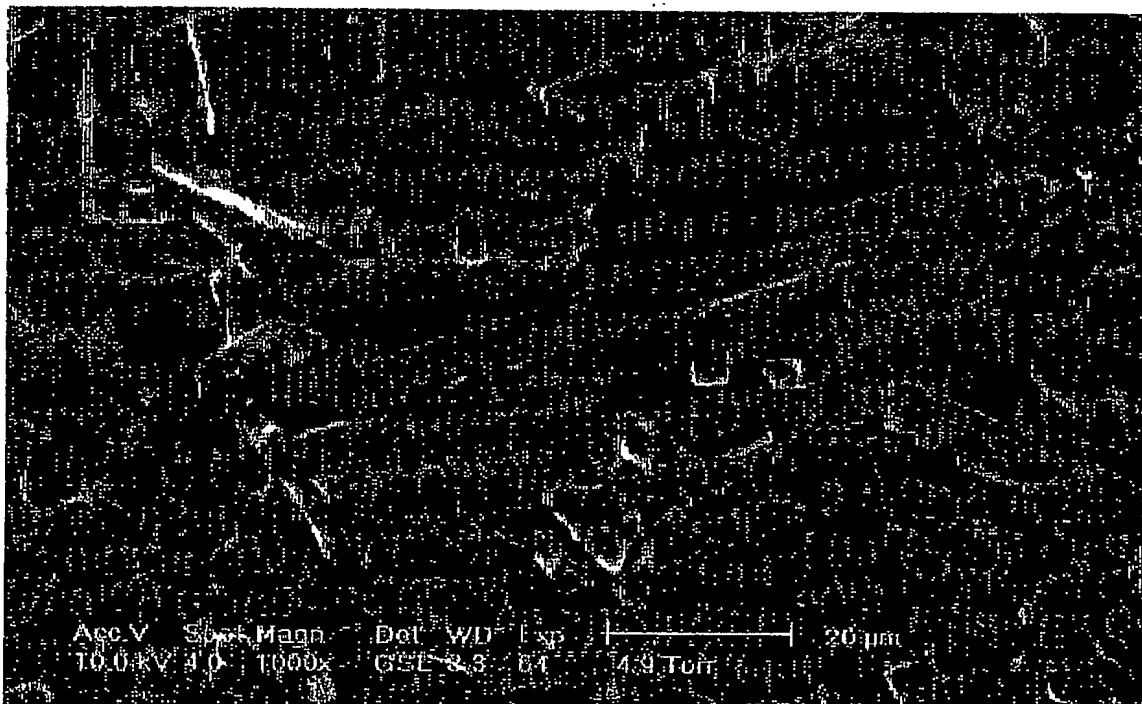


Fig 17

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